

*AMENDMENTS TO THE SPECIFICATION*

Replace paragraph [00105] with:

To identify the human testis-specific CTCF-like protein(s), a variety of oligonucleotides homologous to regions of sequence identity found in the frog, chicken, mouse, rat, and human CTCF cDNAs were designed by the Pile-up and Pretty plot algorithms of the Wisconsin GCG package. Specifically, frog, chicken, mouse, rat, and human CTCF cDNA sequences, as well as *Drosophila* CTCF cDNA (GenBank accession # AF313621; J. Moore, G. Filippova, and V.V.L., unpublished results) were all included in a search for exceptionally conserved short DNA segments for use in designing the PCR-screening primers listed in Figure 4A. These were used in numerous combinations in attempts to PCR-amplify human testis-specific CTCF-like cDNA fragments. As a template, the “MARATHON MARATHON@-Ready” human testis cDNA (Clontech, Palo Alto, CA; cat# 7415-1) was used. Annealing temperatures were adjusted according to the lowest  $T_m$  of the primer in each pair minus 6 °C. Each combination of primer pairs was utilized to work with a ~~MasterAmp~~ MASTERAMP<sup>™</sup> PCR Optimisation Kit (Epicentre Technologies, cat#MO7201). PCR products were analyzed on agarose gels. Distinct DNA-bands were purified and cloned into pCR 2.1-TOPO vector (Invitrogen) and subsequently sequenced. Over a hundred of resulting fragments were cloned into the vector and sequenced. One such fragment displayed a novel human cDNA sequence containing an ORF encoding CTCF-like ZFs. This sequence served to design the new pairs of primers, NEW/TC/for and NEW/TC/rev (Fig. 5A), for a stringent PCR analyses of the “Rapid Screen Arrayed human testis cDNA Library Panel” (Origene), as well as for the 5'- and 3'-RACE with the ~~MARATHON~~ MARATHON@-Ready testis cDNA and adaptor primers from the ~~Marathon~~ MARATHON@ cDNA Amplification kit (Clontech, Palo Alto, CA). This resulted in isolation of a near-full length BORIS cDNA insert in the pCMV6 vector, and of the cDNA sequence shown in Fig. 1A. 5' RACE was performed using ~~GeneRacer~~ GENERACER<sup>™</sup> kit (Invitrogen cat# 45-0079) according to the manufacturers instructions. A similar strategy, but based on the finding of near-identical nucleotide sequences in human BORIS and in the murine CTCF cDNAs was used to design pairs of primers, listed in Figure 4B, for a PCR-mediated screening for the mouse homologue in the ~~MARATHON~~ MARATHON@-ready mouse testes cDNA library (Clontech, Palo Alto, CA). Again, after obtaining and sequencing a fragment encoding the mouse BORIS ZF region, new internal specific primers combined with those from the ~~Marathon~~ MARATHON@ cDNA Amplification kit were utilized to subclone and sequence the 5' and 3' termini of the mouse cDNA. This resulted in the murine BORIS cDNA sequence shown in Fig. 1B that extends to the polyA end, but truncates at the

5'-UTR. Specific methods for 5'-RACE over "difficult" GC-rich region will be used to complete sequence of the 5'-UTR.

Replace paragraph [00114] with:

To verify that the cloned BORIS cDNA encodes the same CTCF-site-binding activity that was initially detected in testis NEs by EMSAs, the clone was used to produce a full-length recombinant BORIS in *Pichia pastoris* yeast as described earlier for CTCF (see, e.g., Quitschke et al., *Nucleic Acids Res*, 28:3370-3378 (2000)). CTCF was purified as originally described by Quitschke et. al. (2000), *supra*, with modifications outlined recently by Vostrov et. al., *J Biol Chem*, 8:ms M109748200 in JBC website (2001). Expression of BORIS in yeast was accomplished using the *Pichia* Expression Kit (Invitrogen Co., Carlsbad, CA) according to the manufacturer's instructions, with chromatography steps similar to those described for CTCF (see, e.g., Vostrov et al. (2001), *supra*). Briefly, BORIS cDNA EcoRI – NotI DNA fragment from the pCVM6/BORIS cDNA was re-cloned into the polycloning site of the pPIC3.5 *Pichia* vector that directs intracellular recombinant protein expression in *Pichia pastoris*. The vectors containing BORIS cDNA were transformed into *Pichia* strain KM71 by electroporation. After growth to preparative quantities (10-15g), *Pichia* cells were homogenized with a ~~Bead-Beater~~ BEAD BEATER™ apparatus (Biospec Products, Inc., Bartensville, OK) in buffer containing 40 mM HEPES, pH 7.6, 2mM MgSO<sub>4</sub>, 1mM EDTA, 10 μM ZnSO<sub>4</sub>, 100mM KCl. Cell debris was pelleted at 5,000 g for 10 minutes and the supernatant was further clarified by centrifugation at 100,000 g for 30 minutes. For use as a non-specific control, wild-type *Pichia* yeast protein extract also was prepared and used as a template for coupled *in vitro* transcription/translation in reticulocyte lysate TnT (Promega). Positive clones were amplified, induced for protein expression and screened for the presence of BORIS by Western blotting. The resulting full-length-BORIS proteins were analyzed in EMSAs side-by-side with testis and liver NEs.